Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 718–726
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The dopamine D3 receptor antagonist NGB 2904 increases spontaneous and amphetamine-stimulated locomotion

Laurel M. Pritchard [a,*], Amy Hauck Newman [b], Robert K. McNamara [a], Aaron D. Logue [a,1], Benjamin Taylor [a,2], Jeffrey A. Welge [c], Ming Xu [e], Jianhua Zhang [d,3], Neil M. Richtand [a,d,f]

[a] Department of Psychiatry, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA
[b] NIDA-Intramural Research Program, Medicinal Chemistry Section, 5500 Nathan Shock Dr., Baltimore, MD 21131, USA
[c] Center for Biostatistical Services, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA
[d] Department of Cancer and Cell Biology, University of Cincinnati College of Medicine, 3125 Eden Ave., Cincinnati, OH 45267, USA
[e] Department of Anesthesia and Critical Care, University of Chicago Medical Center, 5841 S. Maryland Ave., Chicago, IL 60637, USA
[f] Cincinnati Veterans Affairs Medical Center, Psychiatry Service, 3200 Vine St., Cincinnati, OH 45220, USA

Received 4 October 2006; received in revised form 16 February 2007; accepted 26 February 2007
Available online 6 March 2007

Abstract

The dopamine D3 receptor is believed to play an important role in regulation of rodent locomotor behavior, and has been proposed as a therapeutic target for substance abuse, psychotic disorders, and Parkinson's disease. One model of dopamine D3 receptor function, based on studies utilizing D3 receptor knockout mice and D3 receptor-preferring agonists, proposes that D3 receptor stimulation is inhibitory to psychostimulant-induced locomotion, in opposition to the effects of concurrent dopamine D1 and D2 receptor stimulation. Recent progress in medicinal chemistry has led to the development of highly-selective dopamine D3 receptor antagonists. In order to extend our understanding of D3 dopamine receptor's behavioral functions, we determined the effects of the highly-selective dopamine D3 receptor antagonist NGB 2904 on amphetamine-stimulated and spontaneous locomotion in wild-type and dopamine D3 receptor knockout mice. NGB 2904 (26.0 μg/kg s.c.) enhanced amphetamine-stimulated locomotion in wild-type mice, but had no measurable effect in dopamine D3 receptor knockout mice. Of a range of doses (0.026 μg–1.0 mg/kg) given acutely or once daily for seven days, the highest dose of NGB 2904 (1.0 mg/kg) stimulated spontaneous locomotion in wild-type mice, but was without measurable effect in dopamine D3 receptor knockout mice. These behavioral effects of NGB 2904 contrast with those described for other highly D3 receptor-selective antagonists, which have not previously demonstrated an effect on spontaneous locomotor activity. In combination, these data add to the behavioral profile of this novel D3 receptor ligand and provide further support for a role for dopamine D3 receptor inhibitory function in the modulation of rodent locomotion.

Keywords: Dopamine D3 receptor; Knockout mice; Amphetamine; NGB 2904; Locomotor activity

1. Introduction

The effects of dopamine are mediated via two receptor families, the D1 family (D1 and D5) and the D2 family (D2, D3, and D4) (Civelli et al., 1993; Emilien et al., 1999; Missale et al., 1998). Dopamine receptor subtypes differ widely in affinity for dopamine, and therefore in the dopamine concentration range over which receptor occupancy varies and alterations in cellular signaling that occur as a result of receptor activation (Richtand et al., 2001; Richtand, 2006; Vallone et al., 2000). The dopamine D3 receptor has highest affinity for dopamine, and is the only dopamine receptor with a low affinity state K_i within

* Corresponding author. Neuroscience Program, Williams College, 18 Hoxsey St., Bronfman Science Center, Williamstown, MA 01267, USA. Tel.: +1 413 597 3546; fax: +1 413 597 2085.
E-mail address: Laurel.M.Pritchard@williams.edu (L.M. Pritchard).
[1] Current address: Procter & Gamble Co., 1 Procter & Gamble Plaza, Cincinnati OH 45201, USA.
[2] Current address: 1401 Blair Mill Rd., Apt. 1609, Silver Spring, MD 20910, USA.
[3] Current address: Department of Pathology, University of Alabama, Birmingham College of Medicine, 1530 3rd Ave. S., Birmingham, AL 35294, USA.

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.02.019

the concentration range of basal extracellular dopamine levels. The dopamine D3 receptor low affinity state K_i=30 nM (Sokoloff et al., 1992), comparable to estimated basal DA concentrations in rat nucleus accumbens of approximately 3–5 nM in extracellular space (Kalivas and Duffy, 1993; Parsons and Justice, 1992) and 50 nM at the synapse (Ross, 1991). In contrast, dopamine D2 (low affinity state K_i=2 μM) and D1 receptors (low affinity state K_i=2.3 μM) (Sokoloff et al., 1992) require significantly higher dopamine concentrations to achieve 50% occupancy (Richtand et al., 2001). Dopamine D3 receptor mRNA and protein are expressed most abundantly within limbic brain regions associated with modulation of primitive, highly-motivated and emotional behaviors (Landwehrmeyer et al., 1993; Levant, 1998; Levesque et al., 1992; Sokoloff et al., 1990), and D3 receptor protein is expressed on the plasma membrane independently of synaptic boutons (Diaz et al., 2000). Therefore, dopamine D3 receptors are well situated with respect to affinity, extrasynaptic localization, and neuroanatomical distribution to modulate limbic system intracellular signaling through volume transmission, signaling fluctuations in basal dopamine concentrations.

Data from studies using D3 receptor knockout mice support a model in which dopamine D3 receptor stimulation opposes the effects of concurrent D1 and D2 dopamine receptor stimulation at the cellular and systems levels (Xu et al., 1997; Zhang et al., 2004). The behavioral consequences of D3 receptor stimulation, however, have not been unambiguously determined. Evidence from both D3 receptor knockout and pharmacological studies suggest that D3 receptor stimulation inhibits rodent locomotion, in opposition to the behavioral effects of concurrent D1 and D2 receptor stimulation (Pritchard et al., 2003; Svensson et al., 1994; Waters et al., 1993; Xu et al., 1997; Zhang et al., 2004). However, studies analyzing the behavioral effects of D3-preferring antagonists in wild-type and D3 receptor knockout mice observed that both U99194 and nafadotride, compounds with modest D3 receptor selectivity (14–30 and 10-fold selectivity for D3 vs. D2 receptors, respectively), had similar effects on locomotion in wild-type and D3 receptor knockout mice (Boulay et al., 1999; Xu et al., 1999), suggesting that the D3 receptor does not modulate rodent locomotion. Data supporting a facilitating role for the D3 receptor in modulation of rodent locomotion have also been reported (Bordet et al., 1997; Leriche et al., 2003). However, all the studies cited above used D3 receptor knockout mice, compounds with limited D3 receptor selectivity, or both. Therefore, concerns about compensatory adaptations in genetically-altered animals, and the possibility that D3-preferring antagonists actually mediate their effects by blocking D2 receptors, complicate the interpretation of these studies.

Recent progress in medicinal chemistry, however has led to a growing number of more selective D3 receptor antagonists (Austin et al., 2001; Grundt et al., 2005), therefore providing more effective approaches to elucidate D3 receptor behavioral functions. Highly-selective D3 receptor antagonists, including SB-277011-A and S33084 (both 100-fold preference for D3 vs. D2 receptors), did not produce an observable behavioral effect on spontaneous or stimulant-induced locomotion in rats (Millan et al., 2000, 2004; Reavill et al., 2000). In order to further explore D3 receptor behavioral function, we determined the in vivo behavioral effects of another highly D3-selective antagonist (Newman et al., 2005) in both wild-type and D3 receptor knockout mice.

NGB 2904 (*N*-(4-[4-{2,3-dichlorphenyl}-1piperazinyl] butyl)-2-fluorenylcarboxamide), first synthesized and described by Yuan et al., (1998), is a novel compound with in vitro D3 receptor antagonist activity and is structurally similar to the D3-preferring partial agonist/antagonist BP 897. Previous studies have characterized the D3 receptor selectivity of NGB 2904 vs. G protein-coupled receptors with closest sequence homology to the D3 receptor: D2 receptor, D4 receptor, and serotonin 5HT2 receptor (Callier et al., 2003). NGB 2904 has 830-fold selectivity for rat D3 vs. D2 receptor expressed in Sf9 cells (Newman et al., 2003), 155-fold selectivity for cloned primate D3 vs. D2 receptor (Yuan et al., 1998), 60–90-fold selectivity for human D3 vs. D2 receptor (Grundt et al., 2005), and greater than 3500-fold selectivity for D3 vs. D4 receptor (Yuan et al., 1998). NGB 2904 has 160-fold selectivity for rat D3 vs. 5HT2 receptor (Yuan et al., 1998). Additionally, a global receptor screen indicated negligible affinity at other binding sites tested, including α_1 adrenergic receptors (Yuan et al., 1998). Based on prior observations that D3-preferring agonists inhibit rodent locomotion (Ireland et al., 2005; Pritchard et al., 2003; Waters et al., 1993; Xu et al., 1997), we predicted that selective D3 receptor blockade would result in unopposed D1/D2 receptor-mediated signaling (Xu, 1998; Zhang et al., 2004), thereby increasing locomotor activity. We examined the effects of acute and sub-chronic NGB 2904 administration on spontaneous locomotion and of acute NGB 2904 administration on amphetamine-stimulated locomotion in wild-type and D3 receptor mutant mice. Consistent with an inhibitory role of the D3 receptor in rodent locomotion, the D3 receptor antagonist NGB 2904 increased basal and amphetamine-induced locomotor activity in wild-type but not D3 receptor mutant mice.

2. Materials and methods

2.1. Animals

Homozygous wild-type and D3 receptor mutant breeding pairs used to generate the mice used in this study were offspring of mice used in previous studies (Xu et al., 1997). Genetic backgrounds of both mutant and wild-type mice were C57BL/6 × 129 Sv, and had been subsequently bred for three generations with C57BL/6 mice. Genotypes of breeder pairs and all offspring used for experiments were confirmed by PCR as described previously (Pritchard et al., 2003). Mutant and wild-type mice were housed in separate cages, with no more than five animals per cage, under controlled temperature and humidity on a 12-hour light/dark cycle (0500 on; 1700 off). Food and water were available ad libitum. All experiments were performed using adult, 9–16 weeks old (median age for both genotypes: 12 weeks), male wild-type and D3 mutant mice. All experiments were approved by the local Institutional Animal Care and Use Committee and carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral testing equipment

Behavioral testing was performed in 30 custom-designed Residential Activity Chambers. Each chamber consisted of a lighted, ventilated, sound-attenuated cabinet (Cline Builders, Covington, KY) housing a 40×40×38 cm Plexiglas enclosure. A fan in each enclosure provided air circulation and constant background noise. Lights inside the chambers were coordinated with the vivarium light cycle, and behavioral testing was performed during the "lights-on" portion of the cycle (between 0900 and 1700). Locomotion was monitored with a 16×16 photo beam array (San Diego Instruments, San Diego, CA) located 1.25 cm above the floor of the enclosure. Locomotion was expressed as distance traveled.

2.3. Drugs

N-(4-[4-{2,3-dichlorphenyl}-1piperazinyl]butyl)-2-fluorenylcarboxamide (NGB 2904) was synthesized in the Section on Medicinal Chemistry, NIDA-IRP, according to the method of Yuan et al. (1998), and was dissolved by heating overnight to 60 °C in 50% polyethylene glycol 400. d-amphetamine sulfate (Research Biochemicals, Int., Natick, MA) was dissolved in 0.9% saline. Drug concentrations are described as fumarate (NGB 2904) or sulfate (amphetamine) salt.

2.4. Procedures

2.4.1. Experiment 1: acute and sub-chronic effects of NGB 2904

Mice were removed from the vivarium and transported to the testing facility (located in an adjacent building) the day before testing (at ~1500). Subjects were placed in the Residential Activity Chambers and allowed to acclimate for 16–18 h before drug administration, during which time, lab chow and water were available. At ~1000 the following day, food and water were removed from the chambers prior to drug administration, so that feeding behaviors would not compete with locomotor behavior. Mice were injected with one of the following: vehicle (50% PEG 400), 0.026, 2.6, 260.0, 333.0 or 1000 μg/kg NGB 2904. Subjects were then placed back in the Residential Activity Chambers, and locomotor activity was recorded for 2 h. After the two-hour testing period, mice were returned to their home cages overnight. Injections were repeated once daily for 7 days, and the distance traveled during the first 2 h on each day was used to calculate the average distance traveled for the seven-day period. Each animal received only one dose of NGB 2904 on day 1 and continued to receive the same dose on all seven test days.

2.4.2. Experiment 2: effect of NGB 2904 on amphetamine-stimulated locomotion

In a series of pilot experiments, it was determined that NGB 2904 concentrations in the low microgram range increased amphetamine-stimulated locomotion (data not shown). The dose tested in this experiment was derived from these pilot experiments. Mice were acclimated to the Residential Activity Chambers for 1 h, then injected with vehicle (50% PEG 400) or NGB 2904 (26.0 μg/kg). Thirty minutes later, mice were injected with amphetamine (2.5 mg/kg) or 0.9% saline. Locomotor activity was recorded for 3 h after the second injection. We have previously determined that the amphetamine 2.5 mg/kg dose induces primarily elevated locomotor behavior in both wild-type and D3 receptor knockout mice, with negligible competing stereotyped behaviors observed (McNamara et al., 2006). All injections were subcutaneous in a volume of 1.0 ml/kg. Each animal was used for a single behavioral observation, to avoid alterations in locomotor response through the effects of behavioral sensitization. The number of D3 receptor mutant mice available for study limited the experiment to a single NGB 2904 dose.

2.5. Statistical analysis

Data for experiment 1 were analyzed by two-factor ANOVA, with NGB 2904 dose and genotype as between-subjects factors and $\alpha = 0.05$. Data were expressed as arithmetic means of distance traveled for the first 2 h after drug administration, normalized to the group mean for vehicle response. Where main effects were significant, pairs of means were compared using Bonferroni post-hoc tests for multiple comparisons. To determine whether a change in locomotor response to NGB 2904 developed over the seven-day course of treatment, a one-way repeated measure ANOVA was performed in each of the twelve treatment groups (six doses × two genotypes). Day of treatment (with seven levels) was the repeated factor, and the natural logarithm of distance traveled over the observation period was the dependent measure, in order to normalize right-skewing of the distance traveled data as we have previously described (Welge and Richtand, 2002). The first day of treatment was compared to each subsequent day using Dunnett's procedure for multiple comparisons against a common control, in addition to the overall *F*-test for the main effect of treatment day.

Interval data for experiment 2 were analyzed by mixed-model ANOVA, with treatment and genotype as between-subjects factors and time as a repeated, within-subjects factor. This analysis model accounts for the dependence among multiple observations on the same animal, although the primary hypothesis tests are time-averaged contrasts involving the treatment and genotype factors. Specifically, we tested whether (H1) the stimulatory effect of amphetamine was increased in wild-type mice treated with NGB 2904 relative to wild-type mice treated with vehicle, (H2) the stimulatory effect of amphetamine was increased in D3 receptor mutant mice treated with NGB 2904 relative to D3 receptor mutant mice treated with vehicle, and most critically (H3) whether the NGB 2904 effect on amphetamine-stimulated locomotion was significantly larger for the wild-type genotype than the mutant genotype (i.e. whether the effect described by (H1) is larger than the effect described by (H2)). Equality of each of the above expressions to zero was performed with a one-sided test at $\alpha = 0.05$, implemented with the ESTIMATE facility of SAS PROC MIXED, version 8.2.

3. Results

3.1. NGB 2904 increases spontaneous locomotor activity in wild-type but not D3 receptor mutant mice

Acclimated wild-type and D3 knockout mice were treated with a single administration of vehicle or NGB 2904 (0.026 μg/kg–1 mg/kg), and locomotion determined for 2 h following treatment. As shown in Fig. 1A, there was a significant main effect of NGB 2904 dose on locomotor activity ($F_{5,135}=3.632$, $p=0.004$), a significant effect of genotype ($F_{1,135}=13.053$, $p<0.0001$) and a significant interaction between treatment and genotype ($F_{5,135}=3.360$, $p=0.007$). Post-hoc analyses revealed that the highest NGB 2904 dose (1 mg/kg) significantly elevated locomotor activity in wild-type mice ($p<0.0001$). Lower NGB 2904 doses were not measurably different from vehicle in wild-type mice ($p>0.05$). NGB 2904 did not alter locomotor activity in D3 receptor mutant mice at any of the doses tested ($p>0.05$). The magnitude of locomotor activity in response to treatment with 1 mg/kg NGB 2904 was significantly greater in wild-type mice than in D3 receptor mutant mice ($p<0.0001$). This was not true for any of the lower NGB 2904 dosages. Locomotor activity data were also analyzed to test for possible differences in baseline locomotor activity between wild-type and D3 receptor mutant mice. In this study, there were no genotype differences in locomotor response to vehicle injection (data not shown).

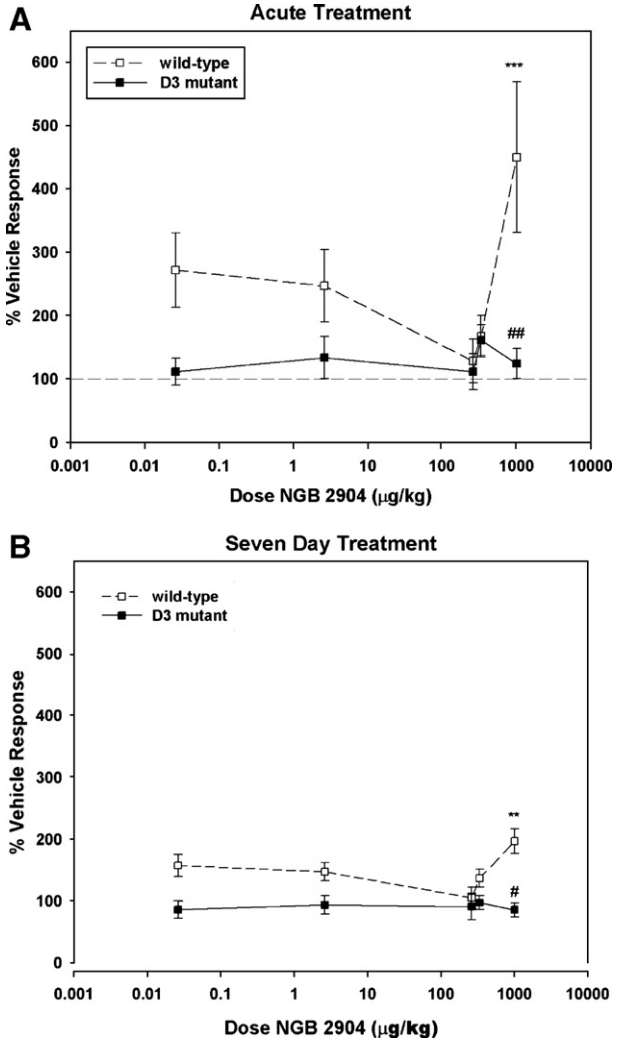

Fig. 1. NGB 2904 stimulates locomotion in acclimated wild-type, but not dopamine D3 receptor mutant mice. Upper panel A: On the first day of treatment (acute response), 1.0 mg/kg NGB 2904 significantly stimulated locomotion in wild-type but not D3 receptor mutant mice. Lower panel B: When distance traveled was averaged over 7 days of testing, 1.0 mg/kg NGB 2904 significantly stimulated locomotion in wild-type but not D3 receptor mutant mice. $^{**}p<0.01$ vs. wild-type vehicle; $^{***}p<0.0001$ vs. wild-type vehicle; $^{\#}p<0.01$ vs. wild-type 1.0 mg/kg; $^{\#\#}p<0.0001$ vs. wild-type 1.0 mg/kg.

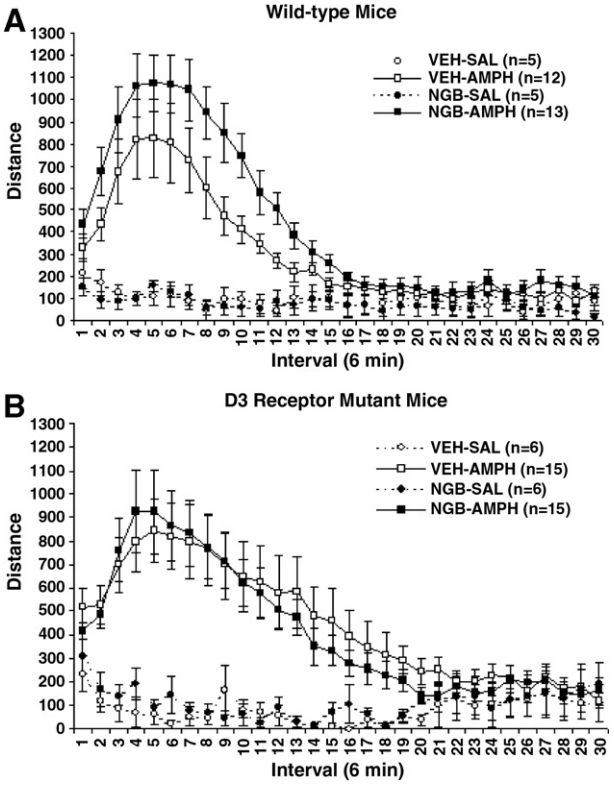

Fig. 2. NGB 2904 enhances amphetamine-stimulated locomotion in wild-type, but not dopamine D3 receptor mutant mice. Amphetamine treatment increased locomotion significantly relative to saline in all four genotype (Wild-Type or D3 Mutant)×pre-treatment (NGB 2904 or saline) groups ($p<0.001$ in each case). Upper panel A, wild-type mice: Amphetamine-stimulated locomotion was greater in wild-type mice pre-treated with NGB 2904 than in wild-type mice pre-treated with vehicle ($t_{(H1)}=2.12$, $p=0.019$). Lower panel B, dopamine D3 receptor knockout mice: Amphetamine-stimulated locomotion did not differ between D3 receptor mutant mice pre-treated with NGB 2904 and mice pre-treated with vehicle ($t_{(H2)}=-1.28$, $p=0.897$). The difference of these effects between genotypes was significant ($t_{(H3)}=2.43$, $p=0.009$).

Acclimated wild-type and D3 knockout mice were treated once daily with vehicle or NGB 2904 (0.026 μg/kg–1 mg/kg)×7 days, and locomotion determined for 2 h following each daily treatment. None of the later treatment days differed significantly from the initial baseline day, nor were any of the twelve overall *F*-tests statistically significant, demonstrating lack of a significant change in locomotor response to NGB 2904 over the seven-day course of treatment with D3 antagonist. The locomotor response to NGB 2904 averaged over the 7 days of treatment is shown in Fig. 1B. There was a significant main effect

of genotype ($F_{1,144}=23.072$, $p<0.0001$), and a significant dose × genotype interaction ($F_{5,144}=2.643$, $p=0.026$). There was no significant effect of dose alone ($F_{5,144}=1.670$, $p=0.146$) (Fig. 2B). The dose × genotype interaction appears to result, to a large extent, from significantly greater locomotor activity following treatment with NGB 2904 1 mg/kg in wild-type mice relative to D3 receptor mutant mice ($p=0.001$). Between genotype comparisons were not significant at other dose levels ($p>0.05$).

3.2. NGB 2904 enhances amphetamine-stimulated locomotion in wild-type but not D3 knockout mice

Acclimated wild-type and D3 knockout mice were pre-treated with NGB 2904 (26.0 μg/kg) or vehicle 30 min prior to treatment with amphetamine (2.5 mg/kg), and locomotor activity determined for 3 h following amphetamine injection. As shown in Fig. 2, amphetamine treatment increased locomotion relative to saline treatment in all four genotype (Wild-Type or D3 Mutant) × pre-treatment (NGB 2904 or vehicle) groups $p<0.001$ in each case). As predicted in Hypothesis (1), the amphetamine effect was greater in wild-type mice pre-treated with NGB 2904 than in wild-type mice pre-treated with vehicle ($t_{(H1)}=2.12$, $p=0.019$). As predicted in Hypothesis (2), the amphetamine effect was NOT greater in D3 receptor mutant mice pre-treated with NGB 2904 than in D3 receptor mutant mice pre–treated with vehicle ($t_{(H2)}=-1.28$, $p=0.897$). Finally, as predicted in Hypothesis (3), the difference of these effects between genotypes was significant, i.e. the NGB 2904 effect on amphetamine-stimulated locomotion was significantly larger for the wild-type genotype than the mutant genotype ($t_{(H3)}=2.43$, $p=0.009$).

4. Discussion

The dopamine D3 receptor has been suggested as a potential therapeutic target for substance dependence (Caine and Koob, 1993; Heidbreder et al., 2005; Newman et al., 2005; Pilla et al., 1999), psychotic disorders (Richtand et al., 2001; Sokoloff et al., 1990, 1992), and Parkinson's disease (Bezard et al., 2003; Joyce, 2001), and as such, elucidating the behavioral effects of D3 dopamine receptor stimulation and blockade is of great interest. Evidence suggests the D3 receptor plays an inhibitory role in the locomotor response to psychostimulants, likely through opposition to D1 receptor-mediated intracellular signaling (Xu, 1998; Xu et al., 1997; Zhang et al., 2004). Until recently, however, the limited in vitro selectivity of D3 receptor antagonists complicated interpretation of in vivo behavioral effects. D3-preferring antagonists, including U99194A (14–30-fold preference D3 vs. D2) (Clifford and Waddington, 1998; Waters et al., 1994), nafadotride (9–10-fold preference) (Audinot et al., 1998; Sautel et al., 1995), (+)-AJ76 (1–6-fold preference), (+)-UH 232 (1–8-fold preference) (Audinot et al., 1998; Millan et al., 2004; Svensson et al., 1986), and PD 152255 (45-fold preference) (Corbin et al., 1998) stimulate spontaneous rodent locomotion. In general, these compounds increase rodent locomotion at low doses and inhibit locomotion at higher doses. This biphasic effect is believed to result from D3 receptor blockade at low doses, with increasing D2 receptor occupancy at higher doses (Levant and Vansell, 1997). D3-preferring antagonists have also been observed to augment the locomotor stimulation induced by amphetamine (Waters et al., 1993) and cocaine (Piercey et al., 1992) at low doses, but inhibit the locomotor response to stimulants at higher doses (Waters et al., 1993).

Not all studies of the behavioral effects of D3-preferring antagonists have supported the validity of this model, however. PD 15225 (44-fold preference D3:D2) *decreased* locomotor activity in amphetamine-treated rats (Corbin et al., 1998), and two studies observed equivalent behavioral effects of D3-preferring antagonists, U99194A and nafadotride, in wild-type and D3 receptor mutant mice, suggesting that the observed behavioral effects are likely mediated by D2 autoreceptors and not by the D3 receptor (Boulay et al., 1999; Xu et al., 1999). Furthermore, descriptions of the behavioral phenotype of D3 receptor mutant mice have been variable. Some (Accili et al., 1996; Xu et al., 1997), but not all (Betancur et al., 2001; Waddington et al., 2001) groups have reported that D3 knockout mice are transiently hyperactive in a novel environment. These conflicting results highlight the need for behavioral characterizations with more selective pharmacological agents. Recent progress in medicinal chemistry (Austin et al., 2001; Grundt et al., 2005; Newman et al., 2003) has provided D3 receptor antagonists with greater selectivity than was achievable in earlier studies. Studies evaluating the behavioral effects of these more selective D3 receptor antagonists in rats, including SB 277011-A (100-fold preference) and S33084 (100-fold preference) did not observe significant effects on spontaneous or stimulant-induced locomotion (Millan et al., 2000, 2004; Reavill et al., 2000). The purpose of our study was to compare the behavioral effects of a highly-selective D3 receptor antagonist in wild-type and D3 receptor knockout mice.

We observed that NGB 2904 enhanced amphetamine-stimulated locomotion in wild-type, but not D3 receptor mutant mice. NGB 2904 (1.0 mg/kg) administered either acutely or once daily for 7 days also increased spontaneous locomotion in wild-type, but not D3 receptor mutant mice, suggesting that the locomotor activating effects are mediated by the D3 dopamine receptor. The observation that NGB 2904 at a dose of 1.0 mg/kg had no effect on basal locomotion in D3 receptor mutant mice and did not produce the biphasic behavioral effects observed with other D3-preferring antagonists (i.e. locomotor stimulation at low doses and inhibition at higher doses) suggests that NGB 2904 at a dose of 1 mg/kg does not elicit significant D2 receptor antagonism. We observed non-significant stimulation of spontaneous locomotion at low doses (0.026–2.6 μg/kg), no effect at moderate doses (260–333 μg/kg) and significant locomotor stimulation at a high dose (1.0 mg/kg). A similarly-shaped dose-response curve has also been reported for NGB 2904 inhibition of cocaine cue-induced reinstatement of drug-seeking behavior (Gilbert et al., 2005). This may reflect the presence of two distinct populations of behaviorally relevant binding sites for NGB 2904. D3 receptor interactions with other proteins, including D2/D3 receptor heterodimers, have previously been reported (Nimchinsky et al., 1997; Scarselli et al.,

2001) and could account for distinct populations of D3 receptor binding sites. However, a non-D3 receptor-mediated mechanism for the observed locomotor stimulation at 1.0 mg/kg cannot be entirely ruled out. Such a mechanism is unlikely, given the absence of locomotor stimulation in D3 receptor mutant mice at this dose, but recent reports of compensatory adaptations in dopamine reuptake in the striatum of D3 receptor mutant mice should be considered when interpreting the present results (Le Foll et al., 2005). Alternative mechanisms underlying the shape of the dose-response curve for augmentation of spontaneous locomotion are possible, and further studies will be needed to clarify this issue.

Amphetamine-induced locomotion was augmented at a lower NGB 2904 dosage (26.0 μg/kg) than was required for significant stimulation of spontaneous locomotor activity (1 mg /kg). However, it is important to note that similarly low doses (0.026 and 2.6 μg/kg) produced robust, though not statistically significant, increases in spontaneous locomotor activity in wild-type, but not D3 receptor mutant mice (Fig. 1A and B). It appears that the locomotor stimulation resulting from low doses of NGB 2904 was below the level needed to achieve statistical significance due to high individual variability and the necessity of correction for multiple comparisons. Pharmacological (Levant et al., 1996; Levant and Vansell, 1997) and D3 receptor knockout (Xu et al., 1997; Zhang et al., 2004) studies have suggested that rodent locomotor activity is modulated by an opposing balance of dopamine signaling through D3 (inhibitory) and D1/D2 (stimulatory) receptors. Therefore differences in the relative degree of D1/D2 receptor stimulation driving locomotion under the different conditions could contribute to the higher NGB 2904 dose needed to produce an effect upon spontaneous locomotion compared to amphetamine-stimulated locomotion. At basal dopamine concentrations in ventral striatum (Parsons and Justice, 1992) D1 and D2 receptors are minimally occupied (Richtand et al., 2001), and complete D3 receptor blockade (high dose antagonist) may be necessary to produce a measurable locomotor increase. In the presence of amphetamine, however, D1 and D2 receptors, as well as D3, are significantly occupied by dopamine (Richtand et al., 2001). Under such conditions, even partial D3 receptor blockade (low dose antagonist) may augment locomotor activity. Another possible explanation for the difference in effective doses of NGB 2904 required to exert a behavioral effect under the two conditions may lie in differences in experimental design. In the evaluation of NGB 2904 effect on spontaneous locomotion, mice were acclimated to the Residential Activity Chambers overnight, whereas in the amphetamine study, mice were acclimated for 1 h. The relative novelty of the testing environment is known to significantly influence behavioral and neural responses to amphetamine (Day et al., 2001). It is therefore also possible that differences in acclimation time between the two experiments could have contributed to the differences in NGB 2904 dose required to exert a behavioral effect. Finally, it is also possible that NGB 2904 affected the pharmacokinetic profile of amphetamine. Given the similar time course of amphetamine-stimulated locomotion in NGB 2904- and vehicle-treated mice, however, this explanation seems unlikely. Additional studies would be needed to unambiguously rule out pharmacokinetic effects.

To assess whether either tolerance or augmented locomotor response to D3 antagonist treatment developed with repeated NGB 2904 administration, we compared the locomotor response on the first treatment day to each subsequent treatment day, and also examined one-way repeated measures ANOVA for a main effect of treatment day. We did not observe any alteration in locomotor response to repetitive NGB 2904 treatment. In contrast, it has been reported that chronic administration of the D3 receptor antagonist SB-277011-A decreases activity of midbrain dopamine neurons (Ashby et al., 2000). Although the behavioral assay employed in our study did not illuminate a behavioral consequence of repetitive NGB 2904 administration, further study would be needed to more definitively elucidate the long-term behavioral adaptation to repetitive D3 receptor blockade.

4.1. Limitations

The results of our study must be interpreted in light of the known methodological limitations of the techniques employed in our experiments. Behavioral differences between D3 receptor knockout and wild-type mice could result from the absence of D3 receptor function in the knockout mice, but might also be the result of compensatory adaptation in D3 knockout mice to the absence of D3 receptor protein; developmental differences between D3 receptor knockout and wild-type mice, unrelated to absence of D3 receptor function on the day of behavioral testing; or the effects of abnormal rearing or social interaction from D3 receptor knockout mothers (Crawley, 1999). Additionally, pharmacological studies are limited by the selectivity of the compounds employed. In contrast with the present findings, studies of other selective D3 receptor antagonists SB 277011A (Reavill et al., 2000) and S33084 (Millan et al., 2000, 2004) reported no elevation of spontaneous locomotor activity. However, it is important to note that these studies were conducted using rats. Indeed, it has been reported that SB 277011A enhances spontaneous horizontal and vertical locomotor activity in mice, but not in rats (Gyertyan and Saghy, 2004). We have recently replicated our finding that NGB 2904 increases spontaneous locomotor activity in DBA/2 mice (McNamara et al., 2006). Collectively, these findings suggest that locomotor activation is not an idiosyncratic effect of NGB 2904, but rather an effect specific to D3 receptor antagonism in mice. Furthermore, the D3 receptor may play different roles in mouse and rat behavior.

It is not known whether the differences between earlier studies, which demonstrated no effect of selective D3 receptor antagonists on spontaneous and stimulant-induced locomotion (Millan et al., 2000, 2004; Reavill et al., 2000), and our findings are a result of differences in experimental design, drug selectivity, species tested, or other factors. Our findings also provide contrast to studies of other behavioral measures demonstrating D3 receptor-mediated facilitation of movements in animal models of Parkinson's disease. For example, sensitization of rotational behavior by levodopa appeared to

be mediated via increased D3 receptor expression in a rat lesion model of Parkinson's disease (Guillin et al., 2001). In a monkey model of Parkinson's disease, D3 receptor expression was positively correlated with symptoms of levodopa-induced dyskinesia and negatively correlated with Parkinsonian symptoms (Bezard et al., 2003; Guigoni et al., 2005), suggesting that D3 receptor also facilitates movement in this system. Similarly, in studies evaluating the D3 receptor role in NMDA receptor antagonist-induced hyperlocomotion, the partial D3 agonist BP 897 and preferential D3 antagonist nafadotride both prevented MK-801-induced locomotor hyperactivity in wild-type, but not D3 receptor mutant mice, suggesting that D3 receptor stimulation mediates *increased* locomotor activity following NMDA receptor blockade (Leriche et al., 2003). In combination, these divergent findings suggest the possibility that the differential effects of D3 receptor blockade on locomotor hyperactivity are due to actions at different D3 receptor populations. Specifically, increased D3 receptor expression associated with dyskinesia-like behaviors in rats occurred in the dorsal striatum, an area of the rat brain where D3 receptor expression is normally very low (Bordet et al., 1997). D3 receptor protein is more abundant in the primate dorsal striatum, relative to the rat (Morissette et al., 1998), and one study demonstrated that repeated treatment with l-DOPA in MPTP-lesioned monkeys elevated D3 receptor binding in the caudate nucleus above levels observed in non-lesioned controls (Guigoni et al., 2005). Therefore, it is possible that stimulation of D3 receptors located in the ventral striatum inhibits locomotor activity, whereas ectopic or over-expression of D3 receptors in the dorsal striatum results in an excessive motor activation. Further studies are needed to propose and test models accounting for these divergent findings.

Taken together, our findings contribute to a growing body of data suggesting that NGB 2904 acts as a selective D3 receptor antagonist in vivo (Gilbert et al., 2005; Xi et al., 2006) and provides a new and useful tool in elucidating the functional role of the D3 receptor. Given the high degree of in vitro selectivity of NGB 2904 and the lack of measurable effect in D3 receptor mutant mice, our findings provide further support for the hypothesis that the D3 receptor plays an inhibitory role in the modulation of certain domains of rodent locomotor behavior. As with any study using knockout mice, our results must be interpreted with caution, as it is possible that differences in drug response between wild-type and knockout mice could have resulted from compensatory adaptations, rather than from deletion of the D3 receptor gene. Based on its demonstrated behavioral effects and in vitro selectivity, NGB 2904 may prove to be a useful instrument for clarifying the role of the dopamine D3 receptor in substance abuse, psychosis, and Parkinson's disease.

Acknowledgements

This work was supported by the Medical Research Service, Department of Veterans Affairs (NMR), DA016778-01 (NMR), DA14644 and DA17323 (MX), NIDA-intramural research program, National Institutes of Health (AHN), a NARSAD Essel Investigator Award (NMR), and a Scottish Rite Schizophrenia Research Fellowship (LMP).

References

Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, et al. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci U S A 1996;93:1945–9.

Ashby Jr CR, Minabe Y, Stemp G, Hagan JJ, Middlemiss DN. Acute and chronic administration of the selective D(3) receptor antagonist SB-277011-A alters activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. J Pharmacol Exp Ther 2000;294:1166–74.

Audinot V, Newman-Tancredi A, Gobert A, Rivet JM, Brocco M, Lejeune F, et al. A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (+)-S 14297, nafadotride, GR 103,691 and U 99194. J Pharmacol Exp Ther 1998;287:187–97.

Austin NE, Avenell KY, Boyfield I, Branch CL, Hadley MS, Jeffrey P, et al. Design and synthesis of novel 2,3-dihydro-1H-isoindoles with high affinity and selectivity for the dopamine D3 receptor. Bioorg Med Chem Lett 2001;11:685–8.

Betancur C, Lepee-Lorgeoux I, Cazillis M, Accili D, Fuchs S, Rostene W. Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D(3) receptor deficient mice. Neuropsychopharmacology 2001;24:170–82.

Bezard E, Ferry S, Mach U, Stark H, Leriche L, Boraud T, et al. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nat Med 2003;9:762–7.

Bordet R, Ridray S, Carboni S, Diaz J, Sokoloff P, Schwartz JC. Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. Proc Natl Acad Sci U S A 1997;94:3363–7.

Boulay D, Depoortere R, Rostene W, Perrault G, Sanger DJ. Dopamine D3 receptor agonists produce similar decreases in body temperature and locomotor activity in D3 knock-out and wild-type mice. Neuropharmacology 1999;38:555–65.

Caine SB, Koob GF. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. Science 1993;260:1814–6.

Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P. Evolution and cell biology of dopamine receptors in vertebrates. Biol Cell 2003;95: 489–502.

Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol 1993;33:281–307.

Clifford JJ, Waddington JL. Heterogeneity of behavioural profile between three new putative selective D3 dopamine receptor antagonists using an ethologically based approach. Psychopharmacology (Berl) 1998;136: 284–90.

Corbin AE, Pugsley TA, Akunne HC, Whetzel SZ, Zoski KT, Georgic LM, et al. Pharmacological characterization of PD 152255, a novel dimeric benzimidazole dopamine D3 antagonist. Pharmacol Biochem Behav 1998;59: 487–93.

Crawley JN. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 1999;835:18–26.

Day HE, Badiani A, Uslaner JM, Oates MM, Vittoz NM, Robinson TE, et al. Environmental novelty differentially affects c-fos mRNA expression induced by amphetamine or cocaine in subregions of the bed nucleus of the stria terminalis and amygdala. J Neurosci 2001;21:732–40.

Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz JC, Sokoloff P. Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. J Neurosci 2000;20:8677–84.

Emilien G, Maloteaux JM, Geurts M, Hoogenberg K, Cragg S. Dopamine receptors—physiological understanding to therapeutic intervention potential. Pharmacol Ther 1999;84:133–56.

Gilbert JG, Newman AH, Gardner EL, Ashby Jr CR, Heidbreder CA, Pak AC, et al. Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D(3) receptors. Synapse 2005;57:17–28.

Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, et al. Novel heterocyclic trans olefin analogues of *N*-{4-[4-(2,3-dichlorophenyl)

piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. J Med Chem 2005;48:839–48.
Guigoni C, Aubert I, Li Q, Gurevich VV, Benovic JL, Ferry S, et al. Pathogenesis of levodopa-induced dyskinesia: focus on D1 and D3 dopamine receptors. Parkinsonism Relat Disord 2005;11(Suppl 1):S25–9.
Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 2001;411:86–9.
Gyertyan I, Saghy K. Effects of dopamine D3 receptor antagonists on spontaneous and agonist-reduced motor activity in NMRI mice and Wistar rats: comparative study with nafadotride, U 99194A and SB 277011. Behav Pharmacol 2004;15:253–62.
Heidbreder CA, Gardner EL, Xi ZX, Thanos PK, Mugnaini M, Hagan JJ, Ashby Jr CR. The role of central dopamine D3 receptors in drug addiction: a review of pharmacological evidence. Brain Res Brain Res Rev 2005;49: 77–105.
Ireland MD, Lowe AS, Reavill C, James MF, Leslie RA, Williams SC. Mapping the effects of the selective dopamine D2/D3 receptor agonist quinelorane using pharmacological magnetic resonance imaging. Neuroscience 2005;133: 315–26.
Joyce JN. Dopamine D3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs. Pharmacol Ther 2001;90:231–59.
Kalivas PW, Duffy P. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J Neurosci 1993;13: 266–75.
Landwehrmeyer B, Mengod G, Palacios JM. Dopamine D3 receptor mRNA and binding sites in human brain. Brain Res Mol Brain Res 1993;18:187–92.
Le Foll B, Diaz J, Sokoloff P. Neuroadaptations to hyperdopaminergia in dopamine D3 receptor deficient mice. Life Sci 2005;76:1281–96.
Leriche L, Schwartz JC, Sokoloff P. The dopamine D3 receptor mediates locomotor hyperactivity induced by NMDA receptor blockade. Neuropharmacology 2003;45:174–81.
Levant B. Differential distribution of D3 dopamine receptors in the brains of several mammalian species. Brain Res 1998;800:269–74.
Levant B, Vansell NR. In vivo occupancy of D2 dopamine receptors by nafadotride. Neuropsychopharmacology 1997;17:67–71.
Levant B, Bancroft GN, Selkirk CM. In vivo occupancy of D2 dopamine receptors by 7-OH-DPAT. Synapse 1996;24(1):60–4.
Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, et al. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin. Proc Natl Acad Sci U S A 1992;89:8155–9.
McNamara RK, Levant B, Taylor B, Ahlbrand R, Liu Y, Sullivan JR, et al. C57BL/6J mice exhibit reduced dopamine D3 receptor-mediated locomotor-inhibitory function relative to DBA/2J mice. Neuroscience 2006;143(1):141–53.
Millan MJ, Dekeyne A, Rivet JM, Dubuffet T, Lavielle G, Brocco M. S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: II. Functional and behavioral profile compared with GR218,231 and L741,626. J Pharmacol Exp Ther 2000;293:1063–73.
Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M. The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. Psychopharmacology (Berl) 2004;174:341–57.
Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
Morissette M, Goulet M, Grondin R, Blanchet P, Bedard PJ, Di Paolo T, et al. Associative and limbic regions of monkey striatum express high levels of dopamine D3 receptors: effects of MPTP and dopamine agonist replacement therapies. Eur J Neurosci 1998;10(8):2565–73.
Newman AH, Cao J, Bennett CJ, Robarge MJ, Freeman RA, Luedtke RR. *N*-(4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl, butenyl and butynyl)arylcarboxamides as novel dopamine D(3) receptor antagonists. Bioorg Med Chem Lett 2003;13:2179–83.
Newman AH, Grundt P, Nader MA. Dopamine D3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. J Med Chem 2005;48: 3663–79.
Nimchinsky EA, Hof PR, Janssen WGM, Morrison JH, Schmauss C. Expression of dopamine D3 receptor dimers and tetramers in brain and in transfected cells. J Biol Chem 1997;272:29229–37.
Parsons LH, Justice JBJ. Extracellular concentration and in vivo recovery of dopamine in the nucleus accumbens using microdialysis. J Neurochem 1992;58:212–8.
Piercey MF, Lum JT, Hoffmann WE, Carlsson A, Ljung E, Svensson K. Antagonism of cocaine's pharmacological effects by the stimulant dopaminergic antagonists, (+)-AJ76 and (+)-UH232. Brain Res 1992;588: 217–22.
Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, et al. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 1999;400:371–5.
Pritchard LM, Logue AD, Hayes S, Welge JA, Xu M, Zhang J, et al. 7-OH-DPAT and PD 128907 selectively activate the D3 dopamine receptor in a novel environment. Neuropsychopharmacology 2003;28:100–7.
Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, et al. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. J Pharmacol Exp Ther 2000;294:1154–65.
Richtand NM. Behavioral sensitization, alternative splicing, and D3 dopamine receptor-mediated inhibitory function. Neuropsychopharmacology 2006;31: 2368–75.
Richtand NM, Woods SC, Berger SP, Strakowski SM. D3 dopamine receptor, behavioral sensitization, and psychosis. Neurosci Biobehav Rev 2001;25: 427–43.
Ross SB. Synaptic concentration of dopamine in the mouse striatum in relationship to the kinetic properties of the dopamine receptors and uptake mechanism. J Neurochem 1991;56:22–9.
Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, et al. Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. J Pharmacol Exp Ther 1995;275: 1239–46.
Scarselli M, Novi F, Schallmach E, Lin R, Baragli A, Colzi A, et al. D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J Biol Chem 2001;276:30308–14.
Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
Sokoloff P, Martres MP, Giros B, Bouthenet ML, Schwartz JC. The third dopamine receptor (D3) as a novel target for antipsychotics. Biochem Pharmacol 1992;43:659–66.
Svensson K, Carlsson M, Carlsson A, Huff RM, Hjorth S, Kling-Petersen T, et al. Behavioral and neurochemical data suggest functional differences between dopamine D2 and D3 receptors. The putatively selective dopamine autoreceptor antagonists (+)-AJ76 and (+)-UH232 stimulate prolactin release in rats. Eur J Pharmacol 1986;263130:2375–432.
Svensson K, Carlsson A, Huff RM, Kling-Petersen T, Waters N. Behavioral and neurochemical data suggest functional differences between dopamine D2 and D3 receptors. Eur J Pharmacol 1994;263:235–43.
Vallone D, Picetti E, Borrelli R. Structure and function of dopamine receptors. Neurosci Biobehav Rev 2000;24:125–32.
Waddington JL, Clifford JJ, McNamara FN, Tomiyama K, Koshikawa N, Croke DT. The psychopharmacology-molecular biology interface: exploring the behavioural roles of dopamine receptor subtypes using targeted gene deletion ('knockout'). Prog Neuropsychopharmacol Biol Psychiatry 2001;25:925–64.
Waters N, Svensson K, Haadsma-Svensson SR, Smith MW, Carlsson A. The dopamine D3-receptor: a postsynaptic receptor inhibitory on rat locomotor activity. J Neural Transm Gen Sect 1993;94:11–9.
Waters N, Lofberg L, Haadsma-Svensson S, Svensson K, Sonesson C, Carlsson A. Differential effects of dopamine D2 and D3 receptor antagonists in regard to dopamine release, in vivo receptor displacement and behaviour. J Neural Transm Gen Sect 1994;98:39–55.
Welge JA, Richtand NM. Regression modeling of rodent locomotion data. Behav Brain Res 2002;128:61–9.
Xi ZX, Newman AH, Gilbert JG, Pak AC, Peng XQ, Ashby CR, et al. The Novel Dopamine D(3) receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. Neuropsychopharmacology 2006;31:1393–405.
Xu M. Unraveling dopamine D3 receptor function in response to psychostimulants using a genetic approach. Ann N Y Acad Sci 1998;844:27–39.

Xu M, Koeltzow TE, Santiago GT, Moratalla R, Cooper DC, Hu XT, et al. Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. Neuron 1997;19:837–48.

Xu M, Koeltzow TE, Cooper DC, Tonegawa S, White FJ. Dopamine D3 receptor mutant and wild-type mice exhibit identical responses to putative D3 receptor-selective agonists and antagonists. Synapse 1999;31:210–5.

Yuan J, Chen X, Brodbeck R, Primus R, Braun J, Wasley JW, Thurkauf A. NGB 2904 and NGB 2849: two highly selective dopamine D3 receptor antagonists. Bioorg Med Chem Lett 1998;8:2715–8.

Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, et al. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. J Neurosci 2004;24:3344–54.